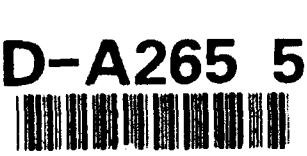

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TITLE: EPIDEMIOLOGICAL SURVEILLANCE AS A BASIS FOR VACCINE TRIALS - ESTABLISHMENT OF A VACCINE EVALUATION UNIT

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Epidemiological Surveillance as a Basis for Vaccine Trials - Establishment of a Vaccine Evaluation Unit

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Approved for public release; distribution unlimited

The broad objectives were to carry out the basic epidemiologic studies necessary for the conducting of vaccine trials. In addition the grant was to be used to set up and maintain laboratory staff and facilities to perform support studies and to conduct clinical trials of vaccines as they become available. It was anticipated that the first vaccines to become available will be a bivalent E. coli-S. flexneri live oral vaccine, a killed hepatitis A vaccine, and vaccines prepared from gram-negative bacteria (Klebsiella, Pseudomonas).

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FOREWORD

The development of new vaccines against shigellosis and hepatitis A, has necessitated the planning and execution of clinical trials of the vaccines. In order to improve the efficiency of these trials, and in order to effectively evaluate their efficacy, it was felt that serologic markers of evidence of previous disease and of susceptibility to future disease should be studied. The current study on the seroepidemiology of shigellosis and hepatitis A was carried out as a collaborative effort between the the Medical Corps of the Israel Defense Force and the Walter Reed Army Institute of Research. For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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INTRODUCTION

Military Importance of the Study and Medical Applications

A number of infectious diseases are of particular importance in military populations. Among them are shigellosis (bacillary dysentery), viral hepatitis and opportunistic gram-negative infections following trauma or burns.

The military importance of shigellosis is well-documented. Despite a decline in the severity of bacillary dysentery over the past few decades, the disease continue to pose a major threat to the operational capability of military units. Although the mortality rate has declined significantly, the substantial morbidity encountered under field conditions in endemic areas such as the Midle East, continue to pose serious operational problems.

The availability of adequate conditions of sanitation and hygiene should be sufficient to prevent epidemic spread of the disease. However, in military populations serving in field units is frequently difficult, if not impossible, to provide such conditions. In such circumstances, effective vaccination may be the only reliable means of preventing outbreaks of the disease.

Israel is a hyperendemic area for shigellosis. Whereas, in USA, 47.4 isolates of Shigella per milion residents were reported in 1980, in Israel more than 1500 cases shigellosis per million residents are reported annually. Thus the reported rate is at least thirty times as high as the reported rate in the USA. In general there is a high rate of shigellosis in the Middle East, particulary amongst young children of nursery-school age where the disease tends to be more severe, with occasional mortality. Thus an effective vaccine may also be useful for preventing the disease in selected pediatric populations.

Hepatitis A is endemic in Israel. The incidence in the general population is relatively high and the IDF uses passive immunization as one of the means of controlling the disease. A vaccine that would induce long-term immunity would be of great value in the control of the disease.

Gram-negative bacteria (e.g. Klebsiella and Pseudomonas) are believed to be responible for much of the wound infection following severe trauma and burns. Both passive and active immunization may provide important tools for control of these infections.

Certain arbovirus infections are endemic in Israel. There are very limited data on the extent of infection due to arboviruses such as West Nile Fever virus or Sandfly viruses among military personnel in Israel.

OBJECTIVES

General

The broad objectives were to carry out the basic epidemiologic studies necessary for the conducting of vaccine trials. In addition the grant was be used to set up and maintain laboratory staff and facilities to perform support studies and to conduct clinical trials of vaccines as they become available. It was anticipated that the first vaccines to become available will be a bivalent E.coli-S.flexneri live oral vaccine, a killed hepatitis A vaccine, and vaccines prepared from gram-negative bacteria (Klebsiella, Pseudomonas).

Since the types of vaccines to be tested vary in nature, it is necessary for the Israel Defense Force to have the capability to perform numerous types of laboratory tests to determine efficacy of the test vaccines. These tests include serological determinations of antibodies, isolation of bacterial and viral pathogens from stool or other clinical samples, and other supporting evaluations as determined by specific protocols.

Specific objectives

1. To study the epidemiology of diarrhea with special reference to shigellosis, using observational, bacteriologic and serologic methods.
2. To study the epidemiology of viral hepatitis using observational and serologic methods.
3. To study the epidemiology of opportunistic infections due to various gram-negative bacteria following trauma and to establish a protocol for a clinical trial to be put into effect on a national basis at the outset of any military conflict.
4. To study the epidemiology of selected arboviruses in Israel.

Additional specific functions of the investigators:

In addition to the performance of epidemiologic studies, the investigators undertook to prepare and review "human use" protocols, summarize and analyze data as collected and participate in annual reviews of the studies in progress.

THE VACCINES

Shigella vaccines

The shigella vaccines to be tested in the projected trials, are based on safe carrier organisms which can express the Shigella type-specific somatic antigen and also invade epithelial cells. Hybrid vaccines against S.flexneri are currently being evaluated in phase 2 studies prior to field-testing.

HEPATITIS VACCINES

A killed hepatitis A vaccine has been produced at WRAIR and underwent safety and antigenicity studies at WRAIR. The vaccine produced by SmithKline Beecham Biologicals was tested for immunogenicity in the IDF.

KLEBSIELLA AND PSEUDOMONAS VACCINES

The HA1A human monoclonal vaccine against gram-negative sepsis was tested in Israel as part of a multi-center study.

STUDY POPULATION CHARACTERISTICS:

The study group for the surveillance of diarrhea consisted of soldiers serving at different sites and undergoing special training programs. They provide cohesive groups which can be maintained under close surveillance relatively easily over a period of several months.

The sero-epidemiologic studies of hepatitis will be carried out on recruits with sera taken at enlistment and on selected subgroups. All clinical cases of hepatitis will be evaluated serologically.

1.0. Task 1 - Basic Epidemiological Surveillance as a Basis for Vaccine Trials - Establishment of Vaccine Evaluation Unit

THE RESEARCH UNIT

A research laboratory was established under the current grant. It covers an area of 200 square meters, and contains facilities for bacteriological, serological and molecular studies, and data processing. An infrastructure for clinical and laboratory surveillance of populations in field units which will be the target of future field trials was also assembled.

OVERALL OBJECTIVES OF THE RESEARCH ACTIVITY

1. Evaluation of vaccines against shigellosis and other diarrheal diseases.
2. Evaluation and improvement of other intervention methods to prevent diarrheal diseases and shigellosis.
3. Evaluation of vaccines against hepatitis A.
4. Evaluation of the exposure of various military populations to arboviral infections.

SHIGELLOSIS, ETEC AND OTHER DIARRHEAL DISEASES

1. Establishment of the logistic framework for carrying out field trials of Shigella vaccines
2. Identification of candidate populations for field trials.
3. Active surveillance of diarrheal diseases in general and shigellosis in particular in military populations serving under field conditions.
4. Identification of the reservoir and the modes of transmission of Shigella organisms in the field.
5. Development of serological markers of the immune status of individuals and populations against shigellosis.
6. Improvement of bacterial isolation techniques under field conditions.
7. Development of a local scheme of S.sonnei phagetyping.

Molecular epidemiology

1. Use of Polymerase Chain Reaction (PCR) and development of group-specific DNA probe to improve sensitivity and for more rapid diagnosis of shigellosis in the field.
2. Use of RFLP in the classification of Shigella organisms.

Assessment of the role played by various immune mechanisms in protection against shigellosis

1. Development of ELISA and HA systems for detection of immune response of individuals to various Shigella antigens such as LPS and OMP.

2. Case-control and prospective studies to evaluate the association between preexposure levels of serum anti-Shigella LPS antibodies and the risk of developing shigellosis due to homologous and heterologous serogroups.
3. Evaluation of the number of antibody secretory cells in the peripheral blood following natural infection with Shigella as an estimate of the immune stimulation of the gut mucosa.
4. Evaluation of the role played by cellular mechanisms of immunity in protection after natural exposure to Shigella spp.

INTERVENTION STUDIES

Evaluation of the impact of intensive fly control on the reduction of the attack rate of shigellosis in field units.

ASSESSMENT OF THE IMPORTANCE OF VARIOUS DIARRHEAGENIC E. coli (E.G. ETEC) IN THE ETIOLOGY OF DIARRHEAL DISEASES AMONG SOLDIERS.

1. Use of synthetic oligonucleotide hybridization probes or PCR for detection of ST and LT of Enterotoxigenic E. coli.
2. Detection of antibody response against the LT of ETEC by using an ELISA system.

HEPATITIS A VIRUS INFECTION

SEROEPIDEMIOLOGICAL STUDIES

Studies of immunity against hepatitis A infection are being carried out in selected samples to evaluate both current and trends in the prevalence of anti-hepatitis A virus antibodies. In addition, data are being collected on characteristics of populations for future vaccine trials.

INCIDENCE STUDIES

A defined population is being maintained under active surveillance for hepatitis (A, B and non-A non-B), infectious mononucleosis and cytomegalovirus infection.

IMMUNOGENICITY STUDIES

A randomized, partly blinded immunogenicity trial of the SKB inactivated hepatitis A vaccine given either alone or concomitantly with immune globulin is near completion.

ARBOVIRAL INFECTIONS

Seroepidemiological studies were carried out in order to estimate the level of exposure of the Israeli population to arboviruses and to assess the role of these agents in the etiology of undiagnosed febrile diseases among soldiers.

JOINT CONFERENCES

In the framework of the grant, joint IDF-US Army annual conferences on vaccines of military importance are held regularly. The first two took place in Israel in 1988 and 1989. The third was held in Washington on June 17-20, 1990, the fourth was held in Israel on October 6th-8th 1991 and the most recent in Anapolis on November 1-6, 1992. Leading researchers in the field of vaccine development and testing from Israel and the United States have participated in these meetings.

2.0. Task 2 - Double-blind, Multicenter, Placebo Controlled Clinical Trial to Evaluate the Efficacy and Safety of HA-1A Human Monoclonal Antibody toPatients with Severe Gram-negative Sepsis/Gram-negative.

Single dose human monolconal antibody against endotoxin (HA-1A) has been shown to decrease mortality in Gram-negative sepsis and bacteremia. To evaluate the effects and safety of multiple doses of HA-1A in clinical sepsis, one or three doses of HA-1A were given to 59 patients with sepsis from November 25, 1990 to October 30, 1991. The study was prospective, randomized and double-blinded. Seven Israeli hospitals participated with an Infectious Diseases specialist and/or intensivist serving as the Principal Investigator at each site.

Enrollment by hospital was:

Hadassah-Hebrew University Medical Center (Jerusalem)	---	21
Central Emek Hospital (Afula)	---	18
Rambam Medical Center (Haifa)	---	8
Beilinson Medical Center (Petah Tikva)	---	5
Chaim Sheba Hospital (Tel Hashomer)	---	5
Soroka Medical Center (Beer Sheva)	---	1
Shaare Zedek Medical Center (Jerusalem)	---	1
Total		59

Thirty six of the 59 patients had Gram-negative infections (61%), 27 had Gram-negative bacteremia (46%), 4 had mixed Gram-negative and positive infections (7%), and 13 of the 59 patients (22%) had no positive cultures (culture negative sepsis).

The overall mortality was 25 of 59 (42%). There were 24 (41%) of the patients with Apache II scores of > 25 with a mortality of 19 of 24 patients (79%). Thirty-five patients (59%) had Apache II scores of $\leq$ 25, with a mortality of 6 of 35 (17%).

Mortality among the patients with Gram-negative infections was 13 of 36 (36.0%) with those patients in shock at baseline in this group having a mortality of 8 of 23 (35%). Mortality among patients with Gram-negative bacteremia was 8 of 27 (30%), and those patients with shock at baseline in this group having a mortality of 5 of 17 (29%).

The primary sites of infection were:

Unknown	32	(55%)
Urinary tract	12	(21%)
Respiratory Tract	6	(10%)
Intraabdominal	5	(7%)
Blood	1	(2%)
Other	3	(5%)

The primary causative organisms of Gram-negative infections were E coli (45%), pseudomonas (20%), Klebsiella (20%), and Enterobacter (15%).

None of the patients had a definite or probable adverse reaction to study medication.

Unblinding of the code and laboratory analyses of blood levels of drug, cytokines, and endotoxin have not been received from Centocor, so that as of March 16, 1993, the laboratory analysis for this study has yet to be performed.

3.0. Task 3 - Shigella Infections in Israeli Military Units

3.1 Surveillance of Shigella Infections in Candidate Populations for Shigella Vaccine Field Trials

The surveillance studies have been carried out in units and populations which are target candidates for the future field trials of Shigella vaccines. These units include young male soldiers, aged 18-21, who undergo relatively long training cycles under difficult field conditions and are extensively exposed to Shigella organisms. Data from the summers of 1988, 1989, 1990 and 1991 surveillance show that 82% to 91% of subjects followed-up during a ten-week cycle of field training, reported at least one episode of diarrhea (2 or more liquid stools per 24 hours) and about 30% of them visited the unit clinic because of diarrhea, during the same period. The overall incidence of shigellosis during the three consecutive summers ranged from 22 to 110 per 1000 per two and a half month period based on Shigella isolates. The incidence of shigellosis broken down by Shigella group ranged as following: S.flexneri (from 2 to 29.0), S.sonnei (from 1 to 76) and S.boydii (from 0 to 72) per 1000 per two and a half month period based on Shigella isolates. Based on recalled diarrhea or visits to clinic due to diarrhea, and serological evidence of infection, the incidences of S.flexneri 2a and S.sonnei shigellosis were much higher ranging from 7.5 to 322 per 1000 and from 36 to 179 per 1000 respectively. Preliminary findings suggest that ETEC could be responsible for an important part of the non-Shigella diarrhea in these field units.

3.2 Fly Control and Attack Rate of Shigellosis

The effect of control of houseflies on the incidence of diarrhea and shigellosis was evaluated in a prospective crossover intervention study on two military field bases several km apart. Beginning in May, 1988 and again in 1989, intensive fly control measures (mainly bait and trap strategy) were introduced in one base, while the other served as a control. After 11 weeks, the intervention was abruptly discontinued in the first base and was instituted in the second for the next 11 weeks. Fly density, incidence of diarrheal disease and of culture-confirmed shigellosis and rates of seroconversion of Shigella and enterotoxigenic Escherichia coli antibodies were measured and compared between intervention and non-intervention bases. Daily fly counts at 1 pm, when houseflies were most dense, were 68% lower on the bases exposed to fly control measures (mean 3.6 vs 11.2, $p = 0.0001$). Concomitantly, clinic visits for diarrheal illness and shigellosis were diminished by 45% (148 cases/1035 soldiers vs 297/1148, $p<0.000001$) and 63% (16/1035 vs 48/1148 soldiers, $p=0.0004$), respectively, on the bases with intensive fly control. Moreover, rates of seroconversion of antibodies to Shigella and enterotoxigenic Escherichia coli were reduced by 66% and 54%, respectively, among trainees on the intervention bases ($p<0.00001$). These observations for the first time provide convincing experimental evidence that houseflies, as mechanical vectors, transmit Shigella and enterotoxigenic E. coli diarrheal infections.

4.0 Task 4A - Identification and Characterization of Candidate Populations for a Trial of Hepatitis A Vaccine

Hepatitis A virus (HAV) infection is highly endemic in Israel and extensive use of immune serum globulin is used to prevent the disease in the military. With the recent advances in the development of the new HAV vaccines, it has become necessary to evaluate the possibility of a vaccine trial in the IDF. In order to identify candidate populations for the trial, a sample of recruits was evaluated for HAV antibodies at the beginning of their three year compulsory service. The prevalence of anti-HAV antibodies in this group was 48.1%. There were marked differences by sex, ethnic origin, education, and a measure of socioeconomic status. The prevalence is higher in those of Eastern origin in both sexes. In both sexes and both ethnic groups the prevalence is higher in those of lower education. In both ethnic groups the prevalence is higher among those of Eastern origin. The prevalence was significantly higher among those of lower socioeconomic status in both ethnic groups. These findings describe basic seroepidemiologic data on populations in whom the new HAV vaccines can be tested.

In order to examine changes in the epidemiology of hepatitis A virus (HAV) infection in Israel during the past decade, a seroepidemiological study was carried out in 1989 in a random sample of 1,153 members of the permanent army, aged 21-30. 59.2% of the males and 54.3% of the females were anti-HAV antibody positive ($p=0.22$). At all ages, the highest prevalence was in those of North African origin, followed by those of Asian, native Israeli and Western origin. There was a marked decline in the prevalence of antibodies in later birth cohorts, (from 74.4% in those born in 1959-60, to 47.8% in those born in 1967-8). Age, ethnic origin, number of siblings, more than two younger siblings and smoking were independently significantly associated with anti-HAV antibodies. Despite an overall decline in family size in later birth cohorts, ethnic differences remain prominent. These findings suggest that when the new active hepatitis A vaccines become available, their use in small children should dramatically reduce the incidence of disease in highly endemic areas by limiting intrafamilial spread of the disease.

5.0 Task 4B - A Trial of the Immunogenicity of Inactivated Hepatitis A Vaccine Administered with Immune Serum Globulin

SmithKline Beecham Biologicals (SBBio) has prepared a purified, highly immunogenic, formaldehyde-inactivated and alum-adjuvanted hepatitis A virus (HAV) vaccine containing the HM-175 strain. The vaccine is administered intramuscularly in a schedule of 0, 1, and 6 months. When rapid immunization is needed, it may be desirable to administer immune globulin (IG) concomitantly with the first dose to provide passive protection until adequate active antibody response has developed. To evaluate the immunogenicity of inactivated HAV vaccine when IG is injected simultaneously with the first dose. Four groups of volunteers were included. Volunteers without antibody to HAV were divided into two groups: 28 in group 1 were given HAV vaccine alone and 34 in group 2 received HAV vaccine simultaneously with 5 ml of IG (Cutter) at the time of the first dose; a third group of 43 subjects immune to hepatitis A received hepatitis B vaccine to compare possible adverse reactions to the HAV vaccine. 12 subjects in group 4 received IG alone. Each 1 ml dose of HAV vaccine contained about 100 ng of antigen or 720 EU. Anti-HAV was determined by means of a commercial assay (Abbott: HAV-EIA), and by a modified ELISA developed by SBBio. Selected sera were tested for neutralizing antibodies. No significant adverse reactions were reported. In groups 1 and 2, one month following the first dose, all had detectable antibodies ($\geq$ 20 mIU/ml) by modified ELISA. Five months following the second dose there was a highly significant difference in positivity rates in the two groups by all methods (75.0% in group 1; 11.8% in group 2, $p < .001$, by standard ELISA at 24 weeks). Two months following the third dose, 28/28 (100%) in group 1 and 33/34 (97.1%) of group 2 were positive. At all blood samplings after immunization, the mean antibody concentration by the modified ELISA method was significantly lower in the group that received IG. Concomitant injection of IG at the time of vaccination with the inactivated HAV vaccine depresses the immune response to the initial doses. Although the booster response to the third dose was good, the mean antibody concentration remained lower in those who received IG. Thus it may be desirable either to avoid giving IG simultaneously with HAV vaccine or if it is done to achieve rapid protection, a further booster vaccine dose may be required to ensure long-lasting immunity. These findings may also have implications for the concomitant injection of IG with other inactivated vaccines

6.0 Task 5 - The Importance of Arbovirus Infections Among Israeli Soldiers

Israel is a country known to be endemic for arbovirus infections such as West Nile fever and Sandfly fever. A seroepidemiological survey was carried out in several different groups. The aim of the study was to determine the past exposure of soldiers in the IDF to arbovirus infection. Random samples of three groups of healthy soldiers aged 18-55 were included. Antibodies to West Nile (WN), Sandfly Sicilian (SFS), and Sandfly Naples (SFN), viruses, were assayed for by an ELISA method. A significant rise in prevalence with age for all three viruses examined was found, ranging from 7.2%, 0, and 2.3%, for WN, SFS, and SFN, viruses, respectively, in soldiers at conscription (average age 18.6) to 43.1%, 23.6%, and 30.7%, respectively, in reserve soldiers (average age 49 yr.). The percentage of seropositives for IgG who were also IgM positive were 0.85%, 7.8% and 2.9%, for WNF, SFS and SFN respectively. 14.4% of the seropositives for WNV were also positive for SFS, as compared with 4.3% among the WNV-seronegatives. Among the seropositives for WNV, 22.7% were positive for SFN, while only 6.5% of WNV seronegatives were positive for SFN IgG antibodies. In the case of seropositives for SFS, 45.6% were positive for SFN, as compared with 6.4% for SFS-seronegatives. It is concluded that the prevalences for WN, SFS, and SFN viruses are high and entomological surveys for the main WN virus vector Culex univettatis and the sandfly viruses vector Phlebotomus papatasi, are recommended to asses their distribution in Israel.

7.0 Task 7 - The Role of Various Mechanisms of Immunity in Protection Against Shigellosis after Natural Exposure to Shigella Organisms

7.1. Natural Immunity to Shigellosis in Groups with Different Previous Risks of Exposure to Shigella

Evidence from human challenge studies and surveillance studies carried out among institutionalized children revealed that clinical shigellosis confers protection of unknown duration against the disease. In Israel, it has been shown that the incidence of the disease may be particularly high among soldiers serving under field training conditions, where the risk of infection with Shigella is increased. In previous case-control and prospective studies we showed that the presence of circulating IgG anti-Shigella lipopolysaccharide (LPS) antibodies is correlated with lower rates of disease. The extent of natural immunity of Israeli soldiers against shigellosis should be associated with the length of previous exposure of this population to field conditions. In this prospective study we hypothesized that the incidence of shigellosis would be lower among soldiers with longer previous service under field conditions. Furthermore, we attempted to determine to what degree this association would be related to higher levels of anti-Shigella LPS antibodies. 695 male subjects aged 18-19 years who had previously served under field conditions for two different periods of time (0 to 6 and 7 to 15 months) and entered a new training course at a camp in a highly endemic area were included to the study. The cohort including these two groups was placed under surveillance for the occurrence of shigellosis for two and a half months, during summer 1989. All subjects were considered to have the same risk of exposure to Shigella and other enteric organisms regardless of previous experience of training under field conditions. Stool samples, data on presence or absence of symptoms and signs of disease, date of onset, and a description of the feces were obtained from each subject complaining of diarrhea at the time of his visit to the unit medical clinic. Blood samples were taken from subjects at the beginning and end of the training period in the field. Additional paired sera were obtained from cases of diarrhea at the acute and convalescent stages of the disease. Isolation and identification of Shigella spp. was performed by routine morphological, biochemical, and serological testing. Immunological tests were carried out using an enzyme-linked immunosorbent assay (ELISA). ELISA was performed in microtitration plates (Costar, Cambridge, MA, US) as previously described. LPS extracted by the method of Westphal from single strains of S.sonnei (form 1) and S.flexneri 2a isolated from outbreaks, were used as antigen. A significant antibody response was defined as a 3-fold or greater increase in the corresponding IgG net optical density (O.D.) where the second serum specimen showed an O.D. of at least 0.25. We defined "pre-existing" anti-LPS antibodies as an OD value of

ELISA IgG equal to or above the cutoff point of 0.20. Chi square and two tailed Fisher's exact tests for differences between percentages were used in the statistical analysis.

The rate of antibody response to Shigella LPS during the two and a half months of follow-up was similar in subjects with short and long previous exposure to field conditions, 55.2% (282/511) and 56.7% (103/184), respectively; this indicates a similar extent of exposure to Shigella organisms during the new training cycle in the field. The incidence of shigellosis defined as visits to the unit clinic because of diarrhea and a significant antibody response against S.sonnei or S.flexneri 2a LPS or both, and/or a positive stool culture for S.sonnei or S.flexneri, was higher among the soldiers with shorter previous service under field conditions as compared to those with longer field experience (10.2% versus 3.8%, respectively) (p=0.008). According to the same definition, the specific incidence rates of S.sonnei shigellosis were 4.5% among subjects with shorter previous service under field conditions as compared to 2.2% among subjects with longer field experience (p=0.16). The specific incidence rates of S.flexneri shigellosis were 8.4% and 3.3% among subjects with shorter and longer previous service under field conditions, respectively (p=0.019). The attack rate of S.sonnei or S.flexneri shigellosis strictly defined as visits to the clinic because of diarrhea (the passage of more than two liquid stools in 24 hr) plus a positive stool culture for these organisms, was also higher among subjects with shorter as compared to those with longer previous exposure to field conditions (3.3% versus 0.05%, respectively) (p=0.05). Out of 18 isolates of Shigella among the subjects under follow-up 11 were S.sonnei and 7 were S.flexneri 2a. The presence of antibodies against S.sonnei and/or S.flexneri 2a LPS at the beginning of the follow-up period was found to be significantly lower among subjects with 0 to 6 months of previous military service under field conditions as compared with subjects that already served between 7 to 15 months under similar conditions (57.9% versus 72.3%, respectively; p=0.001). The association between disease incidence and length of service was further analyzed after stratifying the soldiers according to their initial antibody status. The relative risk of disease with pre-existing antibodies was 3.0, and 1.9 without. By averaging the two groups using the Mantel-Haenszel procedure, the length of previous exposure to field conditions was still significantly associated with the attack rate of shigellosis although to a lesser extent than before stratification (RR=2.3 p=0.023).

This finding indicates that the relationship between the length of exposure to field conditions and the incidence of shigellosis is probably a result of acquired immunity which is only partially reflected in levels of circulating anti-LPS antibodies. Additional immune factors such as secretory IgA and/or occurrence of T-lymphocyte clones probably develop following exposure to Shigella and may have an impact on the incidence of shigellosis.

7.2. IGG Subclass Distribution of the Anti-PS Antibody Response Following *Shigella* Natural Infection.

The IgG subclass distribution of anti-Shigella sonnei and Shigella flexneri LPS antibodies was studied in serum samples obtained from soldiers exposed to these organisms during training cycles under field conditions. Exposure to Shigella was serologically documented by a significant rise in the total IgG antibodies against Shigella LPS in the paired sera collected at the beginning and end of the training cycle in the field. The IgG subclass immune response was determined by an ELISA system using monoclonal antibodies to the four IgG subclasses (IgG1, IgG2, IgG3 and IgG4). We found a different production pattern of IgG subclasses anti-Shigella LPS antibodies after exposure to S.sonnei compared to S.flexneri 2a. IgG1 was found to be the major component in the anti S.sonnei response while anti S.flexneri IgG antibodies are of both the IgG1 and IgG2 subclasses. Out of 117 infected Shigella sonnei patients 81% showed significant increase in the anti-S.sonnei IgG1 subclass antibodies, whereas only 41% of those who were exposed to Shigella flexneri (n=141) showed a significant increase in the anti-S.flexneri 2a IgG1 antibodies. An increase of the IgG2 antibodies to the homologous Shigella LPS was observed in 82.5% and 88% of patients exposed to Shigella sonnei and Shigella flexneri respectively. Twenty seven percent of the subjects which encountered Shigella sonnei infection showed significant increase in the IgG3 subclass, compared to 4% of the patients suffering from Shigella flexneri infections. Complement mediated bacteriolysis of Shigella was associated with high IgG1 but not IgG2 antibody levels against either S.sonnei or S.flexneri. It was also observed that high levels of IgG1 antibodies found in sera of subjects before exposure to either S.sonnei or S.flexneri correlated with high resistance against the disease caused by the corresponding pathogen. It was assumed that serum antibodies homologous to Shigella LPS with a high relative concentration of IgG1 subclass may play an active role in protection against shigellosis.

7.3. Presence of Specific Immunoglobulin A Secreting Cells in Peripheral Blood Following Natural Infection with *Shigella sonnei*

The appearance of antigen-specific IgA antibody secreting cells (ASC) following natural infection with Shigella sonnei during a common source outbreak caused by this organism was evaluated in a modified ELISA assay (ELISPOT). A mean IgA ASC value of $2131.6/10^6$ cells against homologous S.sonnei LPS was detected in blood samples obtained from patients with bacteriologically proven S.sonnei shigellosis 5 and 10 days after the onset of disease. The level of ASC measured in the same blood samples, against heterologous antigen (S.flexneri 2a LPS) was significantly lower then that of the homologous antigen (mean value $33.12/10^6$ cells). Further more, the mean number of activated B cells secreting anti-S.sonnei LPS antibodies was significantly higher among cases of S.sonnei shigellosis as compared to cases of non-Shigella diarrhea ($2.5/10^6$ cells, S.E.=1.0) and "healthy" subjects ($5.1/10^6$ cells, S.E.=2.3) ($p<0.05$). The anti-LPS IgA ASC activity was easily detected within 5 days of onset of disease, a point where the level of anti-S. sonnei LPS IgG and even IgA serum antibodies were hardly detectable.

8.0. Task 8 - Levels of Neutralizing Antibodies to Vaccinia Following Vaccination against Smallpox

The current method for evaluating neutralizing antibodies against vaccinia is the plaque reduction neutralization test. This method is complicated and expensive and is not suitable for large-scale applications. This study was designed to test two methods which may be cheaper and simpler. The first is the microneutralization assay and the second is the ELISA assay. The study group comprised new recruits aged 18-19 years who were inoculated with vaccinia. All had probably been vaccinated against smallpox during infancy. Blood samples were obtained on days 0, 14, 21, 30, 45 and 60, following vaccination. The testing of the sera and analysis of the data are currently being completed.

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APPENDIX I

DEPRESSION OF THE IMMUNE RESPONSE TO AN INACTIVATED HEPATITIS A VACCINE WHEN ADMINISTERED CONCOMITANTLY WITH IMMUNE GLOBULIN

SUMMARY

SmithKline Beecham Biologicals (SBBio) has prepared a purified, highly immunogenic, formaldehyde-inactivated and alum-adjuvanted hepatitis A virus (HAV) vaccine containing the HM-175 strain. The vaccine is administered intramuscularly in a schedule of 0, 1, and 6 months. When rapid immunization is needed, it may be desirable to administer immune globulin (IG) concomitantly with the first dose to provide passive protection until adequate active antibody response has developed. To evaluate the immunogenicity of inactivated HAV vaccine when IG is injected simultaneously with the first dose. Four groups of volunteers were included. Volunteers without antibody to HAV were divided into two groups: 28 in group 1 were given HAV vaccine alone and 34 in group 2 received HAV vaccine simultaneously with 5 ml of IG (Cutter) at the time of the first dose; a third group of 43 subjects immune to hepatitis A received hepatitis B vaccine to compare possible adverse reactions to the HAV vaccine. 12 subjects in group 4 received IG alone. Each 1 ml dose of HAV vaccine contained about 100 ng of antigen or 720 EU. Anti-HAV was determined by means of a commercial assay (Abbott: HAV-EIA), and by a modified ELISA developed by SBBio. Selected sera were tested for neutralizing antibodies. No significant adverse reactions were reported. In groups 1 and 2, one month following the first dose, all had detectable antibodies ($\geq$ 20 mIU/ml) by modified ELISA. Five months following the second dose there was a highly significant difference in positivity rates in the two groups by all methods (75.0% in group 1; 11.8% in group 2, $p < .001$, by standard ELISA at 24 weeks). Two months following the third dose, 28/28 (100%) in group 1 and 33/34 (97.1%) of group 2 were positive. At all blood samplings after immunization, the mean antibody concentration by the modified ELISA method was significantly lower in the group that received IG. Concomitant injection of IG at the time of vaccination with the inactivated HAV vaccine depresses the immune response to the initial doses. Although the booster response to the third dose was good, the mean antibody concentration remained lower in those who received IG. Thus it may be desirable either to avoid giving IG simultaneously with HAV vaccine or if it is done to achieve rapid protection, a further booster vaccine dose may be required to ensure long-lasting immunity. These findings may also have implications for the concomitant injection of IG with other inactivated vaccines.

KEY WORDS: Hepatitis A, vaccine, immune globulin, immunogenicity.

FOREWORD

The development of new vaccines against hepatitis A, has necessitated the planning and execution of clinical trials of the vaccines. The current study on the immunogenicity of the hepatitis A vaccine was carried out as a collaborative effort between the the Medical Corps of the Israel Defense Force and the Walter Reed Army Institute of Research. For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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1.0 BACKGROUND

Work on the development of a vaccine against hepatitis A virus (HAV) infection has been in progress since 1973 when the virus was first observed and subsequently propagated in cell culture (1). The HAV is considered to be a single serotype (2,3) and thus a vaccine should be effective in all parts of the world. Inactivated HAV vaccines were developed by Binn et al (4), Provost et al (5), and others (6), and in early volunteer studies were found to be both safe and immunogenic (7-11). Studies in volunteers have shown a three dose schedule over six months to be as immunogenic as four doses (7). SmithKline Beecham Biologicals (SBBio) has prepared a purified, highly immunogenic formaldehyde-inactivated and alum-adjuvanted HAV vaccine (8). The virus was recovered from the HM-175 strain (12) in certified African green monkey kidney cells and adapted to human diploid MRC-5 cells.

Although final schedules remain to be determined, the vaccine has been administered at 0, 1, and 6 months. Under conditions where vaccinees are due to enter an endemic area before active immunity is present, it may be desirable to administer immune globulin (IG) concomitantly with the first dose to provide protection until adequate antibody response has developed. Recommendations from the United States Centers for Disease Control (CDC) are that live attenuated vaccines should not be given within three months after or less than 2 weeks before receiving IG (13). However, there are no specific recommendations for simultaneous administration of IG with inactivated vaccines such as tetanus, rabies or hepatitis B.

2.0 SPECIFIC AIMS

In this study we wished to determine the effect on the immune response of concomitant administration of IG with the first dose of inactivated HAV vaccine in the proposed three dose schedule.

3.0 METHODS

3.1 Study design

The study was designed as a randomized, placebo-controlled vaccine safety and immunogenicity trial. The study was partially blinded; volunteers did not know if they were receiving hepatitis A or hepatitis B vaccine until the end of the study. The protocol was approved by the Walter Reed Army Institute of Research and Israel Defense Force committees on research with human subjects and all volunteers signed informed consent prior to enrollment.

3.2 Study group

After physical examination, 126 male volunteers aged 19-44 years were enrolled in the study. All subjects fulfilled the inclusion criteria which included healthy general status, normal serum amino-alanine transferase and bilirubin, lack of antibody to HIV and hepatitis B surface antigen. Of the 126 volunteers, 76 were negative for serum anti-HAV antibodies (by commercial Abbott Laboratories HAV-EIA, see below). Those seronegative to HAV were randomized to receive either hepatitis A vaccine alone (Group I) or HAV vaccine and IG (Group II) as described in Table 1. At the end of the study, complete vaccination and follow-up data were available on 28 subjects in group I and 34 subjects in Group II as described in Table 1. In order to evaluate possible adverse reactions to the HAV vaccine, the vaccinees were compared with subjects with detectable anti-HAV antibodies on screening who were given hepatitis B vaccine (Group III). Complete data were available at the end of the study on 43 of the original 50 subjects in this group. The study was single-blinded and subjects in groups I and III did not know whether they received HAV or HBV vaccine. The Group IV volunteers (12 subjects) were recruited separately among subjects who receive IG routinely for pre-exposure prophylaxis purposes and who were negative for serum anti-HAV antibodies.

3.3 Hepatitis A vaccine, hepatitis B vaccine and immune globulin

The hepatitis A vaccine used in this study was manufactured by SBBio, Rixensart, Belgium, as previously described (14). Briefly, the vaccine was prepared from the HM-175 strain from a patient in Australia (12), propagated in MRC-5 cell culture. The virus was purified, formaldehyde-inactivated, alum-adjuvanted and formulated as 720 ELISA units (Eu) per 1.0 ml corresponding to approximately 100 ng of viral antigen. The vaccine was administered by the intramuscular route in the deltoid area at times zero, one and six months. The hepatitis B vaccine (Engerix, SBBio) is commercially available. It is formulated as 10 mcg/ml of recombinant HBsAg per 1.0 ml and contains alum. The vaccine was administered according to the manufacturer's recommendations by the intramuscular route in the deltoid area at times zero, one and six months. The IG was purchased from Cutter Laboratories. It was administered as a single 5.0 ml intramuscular dose in the gluteal muscle. The IG lot used was tested and found to have a 1:2,000 antibody titer and a serum neutralization titer of 2.6 million.

3.4 Clinical monitoring

Each vaccinee was requested to record pre-defined signs and symptoms for 3 days following vaccination. Of interest were local reactions in the deltoid area (soreness, erythema, induration) and systemic findings such as fever, malaise, or headache.

3.5 Serological testing

Sera were obtained prior to the administration of any of the three products used in the study. Following immunization, sera from groups I, II and III were collected at times: 4, 6, 12, 24 and 32 weeks. Sera were collected from group IV at 2, 12 and 24 weeks. One to two months following each vaccine dose, fresh serum specimens were tested for evidence of hepatitis (elevated bilirubin and aminoalanine transferase), by standard laboratory methods. Serum samples were stored at -20°C until tested for antibodies. The antibody studies were performed by several methods. Anti-HAV was determined by means of a commercial EIA assay (HAV-EIA, Abbott Laboratories, North Chicago, IL) at the Israel Defense Force Medical Corps Health Branch Research Laboratory. In addition, a more sensitive ELISA test (14) was carried out at the SBBio laboratories in Rixensart, Belgium. In this test the anti-HAV antibody titer is expressed in milli-international units per ml (mIU/ml) relative to standard World Health Organization reference sera. This test is considered positive when values of $\geq$20 mIU/ml are detected. Randomly selected sera were tested for neutralizing antibody by the RIFIT method as described previously (15). In this test, a titer of $\geq$ 1:10 is considered positive. Anti-hepatitis B surface antigen antibodies were tested by means of ELISA (Abbott Laboratories, North Chicago, IL).

Fisher's two-tailed exact test and the chi-square method were used for statistical comparison among groups and one-way analysis of variance was used to test for differences between means of the logarithmic antibody titers at different points in time. The unpaired t-test was used to test for differences between means in two groups. 95% confidence intervals were computed using the normal distribution for the log antibody titers.

4.0 **RESULTS**

Clinical monitoring of all 117 volunteers did not disclose any unexpected side-effects. Side effects to the HAV vaccine were minimal and except for soreness at the vaccination site, did not differ significantly from those observed in the group who received HBV vaccine (Table 2). No cases of hepatitis were observed.

Results for the sera assayed by the sensitive SBBio ELISA method are presented in Table 3. All volunteers who received hepatitis A vaccine alone or in combination with IG had detectable anti-HAV four weeks after initial immunization. Geometric mean antibody titers were calculated at three different times following immunization (4, 24 and 32 weeks) and are shown in Figure 1 together with the data from group IV who received IG alone. The geometric mean antibody titer for volunteers in Group II was lower at each point when compared to volunteers in Group I

($p < 0.001$). At all examinations, the geometric mean antibody titer for the group who did not receive IG was between two to three times higher than those who received IG. However, at all stages, geometric mean antibody titers were higher than those in the group which received IG alone. As shown in table 3, a greater proportion of volunteers who received vaccine and IG had lower anti-HAV antibody titers at 4 and 24 weeks following immunization as compared with subjects who received vaccine alone.

When sera were tested for anti-HAV antibody by commercial EIA at 12 weeks (8 weeks after the second dose), 64.3% (18/28) in Group I were positive versus 11.8% (4/34) in Group II ($p<0.001$) (detailed data not presented). Comparable differences persisted at 24 weeks (prior to the booster dose): 75.0% (21/28) and 11.8% (4/34) had detectable anti-HAV in groups I and II respectively ($p<0.001$). After the third vaccine dose, 100% of volunteers in group I and 97.1% of volunteers in group II had detectable antibodies, which is similar to that obse ved with the SBBio ELISA.

The sensitive SBBio ELISA detected antibodies in all recipients of IG alone, two weeks after the first dose (Table 4). The geometric mean antibody titer was 98.9 compared with 165.0 at roughly the same time in those who received HAV vaccine alone, and 93.5 for those who received HAV vaccine with IG. Three months after the IG dose only 1 of the 12 vaccinees had detectable antibodies and three more months later, none of them had detectable antibodies. Volunteers who received IG alone had no detectable anti-HAV by the commercial HAV-EIA method at any time after immunization.

The sera following the first and second doses were tested for neutralizing hepatitis A antibody and the results are presented in Table 5. Among those who received HAV vaccine alone, 63.0% (17/27) had detectable neutralizing antibodies one month after the first dose and 92.6% (25/27) two weeks following the second dose. On the other hand, all those who received HAV vaccine and IG all had detectable antibodies one month following injection. The neutralizing antibody GMT's were five times higher among those who received HAV vaccine with IG one month after the first dose. However, after the second dose, the group receiving HAV alone increased ten times whereas the GMT in the group receiving HAV with IG remained almost the same, with the result that those in the HAV vaccine alone group had a GMT more than double that in the group who received IG with the first dose.

Sera tested for anti-HBs in volunteers who received the hepatitis B vaccine showed no departure from previous reports (16). Two months after the second dose, anti-HBs was found in 68.2% of the volunteers, and two months after the third dose in 100% of the volunteers.

5.0 DISCUSSION

The results of this study show that although the antibody response to HAV vaccine was depressed in those who received IG simultaneously with the first dose, the titers achieved in all subjects were satisfactory. In both groups there was about a 12-fold increase in antibody titer after the third dose, and consequently the difference in geometric mean antibody titers between the two groups persisted.

The immune response to several other vaccines administered together with IG has been described both for live attenuated and inactivated vaccines. The live measles vaccine has been given with IG with poorer response (17). However, minimal effect on the immune response to poliovaccine (18,19) or yellow fever vaccine (18) was observed when IG was given 0 to 7 days before vaccination.

As regards inactivated vaccines, the situation is less clear. There appears to be little effect on the immune response of simultaneous administration of hyperimmune IG with inactivated vaccines such as hepatitis B (20,21) and rabies (22) and the recommendations by the United States CDC do not regard simultaneous injection of IG at the time of vaccination to be contraindicated (13). However, the results of this study show that in the case of the inactivated hepatitis A vaccine, simultaneous injection with IG does depress the immune response.

The mechanism through which IG may depress the immune response to an inactivated vaccine has not been described. However, it is likely to be due to binding of the antigen before it can be taken up by the macrophages and presented to the immune system. In general, IG has been found to be highly effective in preventing HAV infection. There is evidence to suggest that IG not only prevents clinical HAV infection, but also suppresses the immune response to the wild HAV. While early investigators suggested a high incidence of subclinical seroconversion against hepatitis A under cover of IG (23), more recent studies indicate that at least within the six months following administration of IG, both clinical and subclinical infection are rare (24-26). Since the half-life of IG is about 24 days (27), this would explain the reduced effect on the second and third HAV vaccine doses.

6.0 CONCLUSIONS

Despite the fact that passive immunization suppresses the immune response to an inactivated hepatitis A vaccine, use of IG simultaneously with the first vaccine dose is not necessarily precluded. However, it is possible that alternative doses and scheduling of both IG and HAV vaccine would result in better long-term protection. Since the vaccine produces good immune

response early in the schedule it may be possible to use IG in much lower doses to provide cover for two to three months until an adequate immune response develops. Alternatively, a larger amount of antigen could be included in the first vaccine dose if it is given at the same time as IG is injected. In summary, the findings in this study are primarily relevant for the use of inactivated hepatitis A vaccines. However, they may have implications for the concomitant injection of IG with other inactivated vaccines.

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9.0 **TABLES**

TABLE 1: Treatment allocation of the 117 volunteers.

	Number	Age range (yr) (Mean)	Vaccines and schedule
Group I	28	19-40 (21.8)	Hepatitis A vaccine at 0, 1, 6 months.
Group II	34	20-44 (21.9)	Hepatitis A vaccine at 0, 1, 6 months plus 5ml IG at time zero.
Group III	43	20-28 (21.0)	Hepatitis B vaccine at 0, 1, 6 months.
Group IV	12	19 (19.0)	5 ml IG at time zero.

TABLE 2. Side-effects reported following vaccination with an inactivated HAV vaccine given without IG compared with vaccination with HBV vaccine.

	Group I HAV vaccine (N = 28)		Group III HBV vaccine (N = 43)		Difference between the two groups*
Dose 1	n	%	n	%	
Sore arm	8	28.6	4	9.3	p = 0.05
Malaise	0	0	3	7.0	NS
Temp > 38	0	0	0	0	NS
Liver enzymes					
SGOT > 52	0	0	1	2.3	NS
SGPT > 45	0	0	0	0	NS
Dose 2					
Sore arm	5	17.9	5	11.6	NS
Malaise	1	3.6	3	7.0	NS
Temp > 38	0	0	0	0	NS
Dose 3					
Sore arm	5	17.9	0	0	p < 0.01
Malaise	0	0	2	4.7	NS
Temp > 38	0	0	0	0	NS

* Fisher's exact test, two-tailed

TABLE 3. Serum antibody titers to hepatitis A virus in mIU/ml based on SBBio sensitive ELISA determinations, relative to time of vaccination, in volunteers who received hepatitis A vaccine alone (Group I) or in combination with serum IG at the first dose (Group II).

Serum antibody titers (mIU/ml)	Time of blood test: 1 m after 1st dose		5 m after 2nd dose		2 m after 3rd dose	
	n	%	n	%	n	%
Group I (HAV alone) (N = 28)						
< 20	0	0.0	0	0.0	0	0.0
20 - 100	4	14.3	2	7.1	0	0.0
> 100	24	85.7	26	92.9	28	100.0
Geometric mean (95% CI)	165.0 (125.2-217.0)		205.9 (172.4-247.2)		2,671 (2100.6-3394.8	
Group II (HAV + IG) (N = 34)						
< 20	0	0.0	2	5.9	0	0.0
20 - 100	18	52.9	16	47.1	1	2.9
> 100	16	47.1	16	47.1	33	97.1
Geometric mean (95% CI)	93.5 (79.8-108.9)		87.7 (66.0-116.7)		943.4 (692.3-1286.9)	
p value[a]	< 0.01		< 0.001		NS	
p value[b]	< 0.001		< 0.001		< 0.001	

a. Comparing seropositivity in the HAV only group with the HAV + IG group.

b. Comparing geometric means in the two groups.

TABLE 4. Serum antibody titers in mIU/ml based on the SBBio sensitive ELISA determinations, in subjects vaccinated with 5ml of IG only, relative to time of vaccination.

Serum antibody concentration (mIU/ml)	Time of blood test					
	2 weeks after vaccine		3 months after vaccine		6 months after vaccine	
	n	%	n	%	n	%
< 20	0	0.0	11	91.7	12	100.0
20 - 100	5	41.7	1	8.3	0	0.0
> 100	7	58.3	0	0.0	0	0.0
Geometric mean titer (95% CI)	98.9[a] (75.2-130.3)		10.8 (10.0-12.8)		10.0 (10.0)	

a. Significantly different from the other times ($p < 0.001$).

TABLE 5. Serum neutralizing antibody titers relative to time of vaccination, in subjects vaccinated with HAV vaccine alone or with IG.

	Time of blood test							
	HAV only (N = 27)				HAV + IG (N = 32)			
Antibody titer	1 m after 1st dose		2 wk after 2nd dose		1 m after 1st dose		2 wk after 2nd dose	
	n	%	n	%	n	%	n	%
< 10	10	37.0	2	7.4	0	0	0	0
10	13	48.2	2	7.4	2	6.3	7	21.9
40	3	11.1	8	29.6	23	71.9	16	50.0
160	1	3.7	10	37.0	7	21.9	8	25.0
≥ 640	-	-	5	18.5	-	-	1	3.1
GMT (95% CI)	10.0 (7.2-13.9)		106.1 (52.5-214.9)		49.7 (38.5-64.1)		45.5 (30.9-67.4)	
p[a]	< 0.001		0.023					
p[b]	< 0.001		0.037					

a. Comparison between the distribution of titers in the two groups.

b. Comparison between the log GMT's.

FIGURE LEGEND

FIGURE 1. Geometric mean antibody titers based on the SBBio ELISA test, for the groups who received HAV vaccine alone and with IG at the time of the first dose, and a group who received a single dose of 5 ml of IG.

APPENDIX II

SOCIODEMOGRAPHIC FACTORS AND THE DECLINING PREVALENCE OF ANTI-HEPATITIS A ANTIBODIES IN YOUNG ADULTS IN ISRAEL. IMPLICATION FOR THE NEW HEPATITIS A VACCINES

SUMMARY

TITLE: SOCIODEMOGRAPHIC FACTORS AND THE DECLINING PREVALENCE OF ANTI-HEPATITIS A ANTIBODIES IN YOUNG ADULTS IN ISRAEL. IMPLICATIONS FOR THE NEW HEPATITIS A VACCINES.

In order to examine changes in the epidemiology of hepatitis A virus (HAV) infection in Israel during the past decade, a seroepidemiological study was carried out in 1989 in a random sample of 1,153 members of the permanent army, aged 21-30. 59.2% of the males and 54.3% of the females were anti-HAV antibody positive (p=0.22). At all ages, the highest prevalence was in those of North African origin, followed by those of Asian, native Israeli and Western origin. There was a marked decline in the prevalence of antibodies in later birth cohorts, (from 74.4% in those born in 1959-60, to 47.8% in those born in 1967-8). Age, ethnic origin, number of siblings, more than two younger siblings and smoking were independently significantly associated with anti-HAV antibodies. Despite an overall decline in family size in later birth cohorts, ethnic differences remain prominent. These findings suggest that when the new active hepatitis A vaccines become available, their use in small children should dramatically reduce the incidence of disease in highly endemic areas by limiting intrafamilial spread of the disease.

Key words: Viral hepatitis, ethnicity, antibodies

FOREWORD

The development of new vaccines against shigellosis and hepatitis A, has necessitated the planning and execution of clinical trials of the vaccines. In order to improve the efficiency of these trials, and in order to effectively evaluate their efficacy, it was felt that serologic markers of evidence of previous disease and of susceptibility to future disease should be studied. The current study on the seroepidemiology of shigellosis and hepatitis A was carried out as a collaborative effort between the the Medical Corps of the Israel Defence Force and the Walter Reed Army Institute of Research. For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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1.0 BACKGROUND

Hepatitis A virus infection is considered to be highly endemic in Israel (1). However, in serosurveys carried out in 18-year-old army inductees in 1977 and 1987, a significant decline in the prevalence of antibodies was observed (2,3), suggesting a decrease in the infection rate in childhood. In both studies, strong ethnic differences in the Jewish population were evident, with those originating from Asia and North Africa having a much higher prevalence than those from Europe and North America (2,3). Recently it has been shown that these ethnic differences may be partly explained by differences in sibship size (4). In addition, based on reported cases, there is evidence that in the Jewish population at least, the age of peak incidence has shifted from 1-4 years to 5-9 years (1).

In view of the recent promising findings in immunogenicity trials of active hepatitis A vaccines (5,6), current epidemiological data on the transmission of the disease and the immune status of populations in highly endemic areas are needed in order to identify target groups for vaccination.

2.0 SPECIFIC AIMS

In this study the prevalence of anti-HAV antibodies and their correlates with sociodemographic and behavioral factors were examined in a sample of young adults in the permanent army in Israel.

3.0 MATERIALS AND METHODS

Subjects in a random sample of 1,885 males and females aged 21-30 serving in the permanent army in Israel in 1989 were approached to undergo testing for hepatitis A antibodies. A total of 1,153 (61%) responded. There were no major differences between responders and non-responders, although responders tended to be less educated. A questionnaire was administered in which demographic data including age, country of birth, ethnic origin, number of siblings, birth order, (number of persons per room in the household (crowding index) at age 10 years, years of education, years of service in a military field unit, smoking habits, and past history of clinical hepatitis infection were obtained. Ethnic origin was classified according to the fathers birthplace (or where this was Israel, the paternal grandfather's birthplace). This definition is consistent with other Israeli studies of ethnic differences in the prevalence of anti-hepatitis A antibodies. Four broad areas of origin, were defined. N Africa, Asia (mainly the Middle East), West (Europe excluding Turkey, the Americas, Australasia and Southern Africa) and Israel. Sera were

separated from the whole blood and frozen at -20°C until tested. Presence of anti-hepatitis A virus antibodies was determined by means of ELISA (HAVAB, Abbott Laboratories, N. Chicago, IL).

The chi-square tests for differences between percentages and the Mantel-Haenszel test for trend (7) were used to evaluate statistical significance in the univariate analyses. Multiple logistic regression analysis, a technique similar to multiple linear regression analysis and applied when the dependent variable is dichotomous, was used to determine correlates of anti-hepatitis A antibodies while controlling for other potentially confounding factors. One-way analysis of variance was used to determine changes in sibship size and crowding index with later cohorts.

4.0 RESULTS

The distribution of anti-hepatitis A antibody status by region of origin and sex is given in table 1. Overall 59.2% of males and 54.3% of females were positive but the sex differences were not significantly different after controlling for ethnic origin. The prevalence of anti-hepatitis A virus antibodies by ethnic origin varied from a high of 75.6% in those originating from North Africa to 41.6% in those of Western origin. The distribution by region of origin and birth cohort, is given in Table 2. There was a significant and consistent decreasing prevalence with later cohorts in those of Asian and Western origin (87.8% to 51.9% and 56.4% to 36.5% respectively), and a less consistent decline for those of N. African origin. No significant change was observed for those of Israeli origin but the sample size was small with consequently low statistical power. Only 9 (0.8%) of the subjects reported suffering clinical hepatitis over the age of 21 years (2 of N. African origin, 6 of Western origin and 1 originating in Israel). A comparison by ethnic group between the cohort 1959-62 and 1965-7 is shown in Figure 1 and the marked decline in the prevalence of anti-hepatitis A virus antibodies over the period is evident for those of Asian and Western origin (82% to 57% and 58% to 38% respectively) and less so for those originating in North Africa (82% to 73%).

The prevalence of anti-hepatitis A virus antibodies by number of siblings is shown in Table 3. There was a highly significant increase with more siblings in all ethnic groups. A weaker positive association was found with crowding index at age 10 years after controlling for ethnic origin (Table 4). A weaker but significant positive association was found with number of younger siblings and a negative association with education (detailed data not shown). Average sibship size and housing density by ethnic origin and birth cohort are shown in Figures 2 and 3 (the sample of those whose paternal grandfather was born in Israel was too small to be analyzed). Sibship size declined most markedly in

those of Asian origin followed by those of North African origin with no significant change in those of Western origin. The crowding index declined in all three groups, but this was most evident in those of Asian origin.

Results of the multiple logistic regression analysis are shown in table 5. Compared with those of Western origin, the adjusted prevalence in North Africans was nearly 2.4 times higher and that in those of Asian origin, 1.7 times higher. Within the same ethnic group, there was no significant difference between those born in Israel and those born abroad. Age and sibship size were strongly positively associated with anti-hepatitis A virus antibodies. More than two younger siblings and smoking were weaker but statistically significant positive correlates. There was no independent association with education or crowding index.

5.0 **DISCUSSION**

The results of this study provide evidence of a consistent, marked decline in the prevalence of anti-hepatitis A virus antibodies in young adults in Israel over the past decade. The question of whether the apparent cohort effect is actually due to sub-clinical infections occurring over the age 21 is highly unlikely since few of the subjects in the present study could recall clinical hepatitis during adulthood. In order to attribute the cohort effect observed to subclinical infection, this would require a sub-clinical to clinical ratio of much more than 10 to 1, whereas previous studies indicate that in adults, it is no more than about 2 to 1 (8). Thus the increase by age in the study population is most likely to be due to a true cohort effect. The cohort effect for hepatitis A infection has been described in a number of European countries and Australia (9,10). In the present study, the ethnic differences in the Jewish population have persisted. However, the pattern has changed somewhat, since the greatest decline has been in those of Asian and Western origin, with those from North Africa showing almost no change.

Previous investigators have suggested that ethnic differences in the prevalence of anti-hepatitis A antibodies among young adults in Israel reflect, at least partly, variation in socioeconomic status and may also result from some genetic difference in susceptibility to infection (2). However in the present study, the previously described association of anti-hepatitis A virus antibody positivity with sibship size in Israel (4) remained highly significant in all ethnic groups. The decline in family size during the period under study was most evident among those of Asian and North African origin, whereas the crowding index at age 10 declined in all ethnic groups. These changes paralleled the decline in anti-hepatitis A virus antibody prevalence. The fact that among Westerners the decline in the prevalence of anti-hepatitis A antibodies was not associated with

a concomitant decline in family size runs somewhat counter to the trends in the other groups. Since Westerners had a much lower incidence of hepatitis A virus infection during the period under study, it is possible that the decline in antibody prevalence in this group was related more to a general decline in childhood infection in the other ethnic groups, reducing the exposure at places of contact such as day-care centers. In general, the findings in this study support previous observation (4) that the ethnic differences in the risk of infection appears to be related to the presence of young children in the household regardless of socioeconomic status and declining family size will contribute to a reduction in childhood infection.

In countries where there is a high incidence of disease, childhood infection is common and siblings are likely to be an important source of infection to one another (11,12). Even in highly endemic areas infection of susceptible adult visitors appears to be much less than the intrafamilial spread (13). Hepatitis A virus infection is largely asymptomatic in young children, a condition which facilitates transmission since appropriate isolation cannot be implemented (14). The disease is relatively common in employees of day-care centers and household contacts of children aged 1-2 years attending such centers (15,16). Similarly, the larger the family, the greater the likelihood that an asymptomatic infected child in the household will infect the other siblings, particularly where the older children assist in caring for the younger siblings enhanced. In fact, the ethnic differences in the prevalence of hepatitis A antibodies in Israel only become evident after the age of about 5 years (17), further supporting the thesis that infection is more likely to be acquired from younger siblings.

In several studies in Israel, prevalence of anti-hepatitis A antibodies has been found to be negatively related to measures of socioeconomic status (2,3) and improved socioeconomic status is generally accompanied by a reduction in family size. Thus the association between the decline in the prevalence of anti-hepatitis A virus antibodies and the reduction in family size may be confounded by the general improvement in living conditions. However, in the population investigated in the present study, it is highly unlikely that a significant number of the subjects have lived under conditions of compromised sanitation. All were children during the 1960's and during that period housing conditions in this population were generally highly satisfactory. In addition, in the present study most subjects were born in Israel (85%), and thus could not have acquired the disease in their countries of origin. It is also noteworthy that the association of anti-hepatitis A antibodies with country of origin was similar whether or not the subject was born in Israel or abroad.

The ethnic differences in the prevalence of anti-hepatitis A virus antibodies persisted after controlling for measures of socioeconomic status such as education and crowding. This phenomenon contrasts with findings in other parts of the world, where racial differences have not been observed after taking into account socioeconomic status (18). Thus the spread of hepatitis A virus infection in Israel still appears to occur at different rates in the various ethnic groups after controlling for a number of potential confounding factors related to socioeconomic status. The finding of a positive association between cigarette smoking and the presence of anti-hepatitis A virus antibodies after adjusting for the other potential confounders is not easily explained. However, one possibility is that it is simply another marker of lower socioeconomic status.

6.0 **CONCLUSIONS**

The findings in the present study provide evidence of a marked decline in the prevalence of anti-hepatitis A virus antibodies in young adults in Israel during the past decade, although this has not occurred equally in the ethnic groups. This decline may be related, at least partly, to a decrease in family size in Israelis of Asian and North African origin, reducing the risk of infection both from younger siblings in the home and between children of different ethnic backgrounds attending the same day-care centers. These findings suggest that if the new active hepatitis A virus vaccines currently being tested prove to be effective, and will be used routinely in small children, their greatest impact could be in reducing a major source of infection to siblings, playmates and adult contacts.

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9.0 TABLES

TABLE 1. Prevalence of anti-hepatitis A antibody by sex and ethnic origin* in an adult population in Israel

	Ethnic origin							
	North Africa		Asia		Israel		West	
Age	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.
Males	290	76.9	256	65.6	63	52.4	364	41.8
Females	54	68.5	44	56.8	6	66.7	71	40.9
Total	344	75.6	300	64.3	69	53.6	435	41.6
X^2	1.73		1.27		0.45		0.02	
p-value	0.188		0.260		0.503		0.886	

* By father's country of birth or paternal grandfather if father was born in Israel.

** Mantel-Haenszel chi-square for sex differences after controlling for ethnic origin = 1.438, p = 0.230.

TABLE 2. Prevalence of anti-hepatitis A antibody by birth cohort and ethnic origin* in an adult population in Israel.

	Ethnic origin							
	North Africa		Asia		Israel		West	
Birth cohort	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.
1959-60	65	84.6	49	87.8	11	45.5	55	56.4
1961-62	69	76.8	52	69.2	11	81.8	62	51.6
1963-64	55	67.3	44	63.6	11	45.5	88	39.8
1965-66	93	80.7	101	57.4	22	50.0	115	35.7
1967-68	62	64.5	54	51.9	14	50.0	115	36.5
Total	344	75.6	300	64.3	69	53.6	435	41.6
X^2 (M-H trend)		3.8		16.6		0.3		8.8
p-value		0.052		< 0.001		0.585		0.003

* By father's country of birth or paternal grandfather if father was born in Israel.

TABLE 3. Prevalence of anti-hepatitis A antibody by number of siblings and ethnic origin* in an adult population in Israel

	Ethnic origin							
	North Africa		Asia		Israel		West	
No. of siblings	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.
≤ 2	23	52.2	25	56.0	14	14.3	153	32.7
3	43	55.8	61	45.9	23	52.2	174	46.0
4	57	77.2	64	56.3	10	50.0	70	42.9
≥ 5	221	81.5	150	76.7	22	81.8	38	55.3
Total	344	75.6	300	64.3	69	53.6	435	41.6
X^2 (M-H trend)		17.5		12.9		14.6		7.9
p		< 0.001		< 0.001		< 0.001		0.005

* By father's country of birth or paternal grandfather if father was born in Israel.

TABLE 4. Prevalence of anti-hepatitis A antibody by ethnic origin* and crowding index** in an adult population in Israel.

	Ethnic origin							
	North Africa		Asia		Israel		West	
Crowding index	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.	No. tested	% pos.
0.0-1.5	76	67.1	85	60.0	35	40.0	275	39.3
1.5-2.0	71	70.4	68	57.4	17	64.7	95	41.1
2.0-2.5	101	82.2	76	71.1	7	71.4	34	52.9
≥ 2.5	95	79.0	69	71.0	9	77.8	21	57.1
Total	343	75.5	298	64.8	68	54.4	425	41.7
X^2		5.0		3.0		6.3		3.3
p-value		0.025		0.081		0.012		0.071

* By father's country of birth or paternal grandfather if father was born in Israel.

** Number of persons per room in household at age 10 years.

TABLE 5. Variables associated with the presence of anti-hepatitis A antibodies in multiple logistic regression analysis in an adult population in Israel.

Variable	O.R.	95% C.I.	Chi-square	P
Sex (males=1)	1.02	0.72 - 1.46	0.0	0.904
Age (years)	1.11	1.06 - 1.17	16.5	< 0.001
North Africa*	2.36	1.64 - 3.39	21.4	< 0.001
Asia*	1.72	1.23 - 2.43	9.8	0.002
Israel*	1.23	0.71 - 2.13	0.5	0.469
Birth in Israel	1.26	0.87 - 1.83	1.4	0.230
No. of siblings	1.17	1.08 - 1.27	14.8	< 0.001
$\geq$ 2 younger siblings	1.53	1.08 - 2.18	5.7	0.017
Crowding index at 10**	1.02	0.84 - 1.24	0.3	0.864
Education (years)	1.04	0.68 - 1.60	0.0	0.855
Smoking	1.37	1.04 - 1.79	5.0	0.025
$\geq$ 5 years in field unit	1.06	0.69 - 1.62	0.6	0.804

* By father's country of birth or paternal grandfather if father was born in Israel (Westerners are the reference group).

** Number of persons per room in household at age 10 years.

FIGURE LEGENDS

FIGURE 1. Prevalence of anti-hepatitis A virus antibodies by ethnic origin and birth cohort in young adults in Israel examined in 1989.

FIGURE 2. Mean number of siblings by ethnic origin and birth cohort in young adults in Israel examined in 1989.

FIGURE 3. Crowding index by ethnic origin and birth cohort in young adults in Israel examined in 1989.

10.0 **QUESTIONNAIRE**

Demographic information

1. Age !__!__!
2. Sex !__!
3. Country of birth !__!__!__!
4. Father's country of birth !__!__!__!
5. Paternal grandfather' country of birth !__!__!__!
6. Years of education !__!__!
7. Measure of socioeconomic status !__!__!

Laboratory results

Anti-HAV antibodies 1. Positive 2. Negative !__!

APPENDIX III

SEROPREVALENCE STUDY OF HEPATITIS A VIRUS AMONG MILITARY RECRUITS IN THE ISRAEL DEFENSE FORCES

SUMMARY

Hepatitis A virus (HAV) infection is highly endemic in Israel and extensive use of immune serum globulin is used to prevent the disease in the military. With the recent advances in the development of the new HAV vaccines, it has become necessary to evaluate the possibility of a vaccine trial in the IDF. In order to identify candidate populations for the trial, a sample of recruits was evaluated for HAV antibodies at the beginning of their three year compulsory service. The prevalence of anti-HAV antibodies in this group was 48.1%. There were marked differences by sex, ethnic origin, education, and a measure of socioeconomic status. The prevalence is higher in those of Eastern origin in both sexes. In both sexes and both ethnic groups the prevalence is higher in those of lower education. In both ethnic groups the prevalence is higher among those of Eastern origin. The prevalence was significantly higher among those of lower socioeconomic status in both ethnic groups. These findings describe basic seroepidemiologic data on populations in whom the new HAV vaccines can be tested.

KEY WORDS: Hepatitis, antibodies, ethnicity, epidemiology

FOREWORD

The development of new vaccines against shigellosis and hepatitis A, has necessitated the planning and execution of clinical trials of the vaccines. In order to improve the efficiency of these trials, and in order to effectively evaluate their efficacy, it was felt that serologic markers of evidence of previous disease and of susceptibility to future disease should be studied. The current study on the seroepidemiology of shigellosis and hepatitis A was carried out as a collaborative effort between the the Medical Corps of the Israel Defense Force and the Walter Reed Army Institute of Research. For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46. Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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1.0 BACKGROUND

Hepatitis A virus (HAV) infection is highly endemic in Israel with an incidence about ten times as high as that of the United States (1-3). By the age of 18 years, more than 50% of the population have anti-HAV antibodies (4) as compared with about 20% in the USA, indicating that there is a high rate of infection during childhood. Ethnic differences in the prevalence of anti-HAV antibodies within the Jewish population have been described, with those originating from Asia and Africa having a much higher prevalence than those from Europe and North America (4-6). These ethnic differences have in part been ascribed to variations in socioeconomic status, although they persist even after controlling for education level (4-6). This phenomenon contrasts with findings in other parts of the world, where racial differences have not been observed after taking into account socioeconomic status (7-8). Thus factors other than socioeconomic status may account for the ethnic differences in hepatitis A virus infection in Israel. There is a declining trend in the incidence of the disease among children and the proportion of the adult population which is susceptible is increasing (4). Consequently the problem of HAV infection in the military population may well increase in the future.

Studies in the Israel Defense Force have shown HAV infection to be the cause of more than 80% of all clinical cases of infectious hepatitis (9). Passive immunization with immune serum globulin (ISG) is used liberally in the military, both as post-exposure prophylaxis (10,11) and, in a large section of this population, as annual pre-exposure prophylaxis (12). Controlled studies have demonstrated that this policy is highly effective in reducing the incidence of the disease among soldiers and markedly reduces the occurrence of epidemics of hepatitis (12).

An inactivated whole virus vaccine is currently undergoing laboratory and safety tests (13). Such a vaccine would clearly be of great value in the prevention of the disease in the IDF and would eliminate the need for repeated immunization with ISG.

2.0 SPECIFIC AIMS

Objectives

1. To determine the prevalence of anti-hepatitis A virus antibodies in subpopulations of conscripts in the IDF.

2. To determine demographic characteristics associated with differing prevalence of anti-HAV antibodies among recruits.

3.0 METHODS

3.1 Study design

Prevalence study.

3.2 Study population characteristics

Blood samples are routinely drawn from a random sample of recruits each year and the serum stored at -20^{o} C. A sample of soldiers conscripted in 1987/8 were selected from this standard sample and their blood samples were used to determine prevalence of anti-HAV antibodies.

3.3 Sample size

800 soldiers conscripted in 1987-8

3.4 Hepatitis serology

The antibody studies were performed at the central IDF laboratories. Sera was separated from the whole blood and frozen at -20^{o} C until tested. Qualitative evaluation of anti-HAV antibodies was determined by means of solid phase radioimmunoassay (HAVAB - Abbott Laboratories, N. Chicago, IL).

3.5 Interview data

Data available included age, sex, country of origin, education, and a measure of socioeconomic status. Country of origin was defined by the fathers birth place (or where this was Israel, the paternal grandfathers birthplace). Three broad areas of origin, Israel, East and West, were defined. West included Europe (excluding Turkey), the Americas, Australasia and Southern Africa. All others countries excluding Israel (mainly from the Middle East and North Africa) were classified as East.

3.6 Statistical analysis

The chi-square tests for difference between percentages and for trend were used to evaluate statistical significance in the univariate analysis. Multiple logistic regression analysis, a technique similar to multiple linear regression analysis when the dependent variable is dichotomous, was used to determine correlates of anti-HAV antibodies while controlling for other potentially confounding factors.

4.0 RESULTS

The distribution of anti-HAV antibody status at conscription by age and sex, is presented in Table 1. The prevalence of anti-HAV antibodies at conscription was 48.1% and was significantly higher higher in males (51.4%) than females (44.2%). The prevalence by sex and ethnic origin is presented in Table 2. The prevalence is higher in those of Eastern origin in both sexes. The prevalence by ethnic origin and years of education in males is presented in Table 3 and that for females in Table 4. In both sexes and both ethnic groups the prevalence is higher in those of lower education. In both ethnic groups the prevalence is higher among those of Eastern origin. The prevalence by sex and a measure of socioeconomic status is presented in Table 5. The prevalence was significantly higher among those of lower socioeconomic status in both ethnic groups.

The association of the different variables with the prevalence of anti-HAV antibodies while controlling for the effects of the others in multiple logistic regression analysis is presented in Table 6. The measure of socioeconomic status emerges as the strongest predictor.

5.0 DISCUSSION

This study demonstrated that the proportion of susceptibles to HAV infection in selected groups of recruits is high. There has been a decline in the prevalence in anti-HAV antibodies since the last survey in 1977 (11) and slightly lower than that observed in 1984 (4). In the 1984 survey (4), the prevalence of anti-HAV antibodies in the IDF was 54.0%. The prevalence among those of Western origin was 28.0% and the prevalence among those of Eastern origin was 71.1%. When broken down into groups according to educational status the prevalence was lower in the higher educational categories in both large ethnic groups.

We have previously described an association of anti-HAV antibody positivity with sibship size (6). This may partly be explained by transmission of the disease among children. The fact that hepatitis A virus infection is largely asymptomatic in children (14), makes its transmission to close contacts more likely, since appropriate isolation would not be carried out. It has been noted that hepatitis A virus infection is proportionately more common in employees of day-care centers and household contacts of children (particularly diapered) aged 1-2 years at daycare centers (15,16). Thus in large families there is an increased likelihood that there will be an asymptomatic infected child in the household, with the attendant risk of spread to the other siblings. Where the older children assist in caring for the younger children the risk of infection may be considerably enhanced.

6.0 CONCLUSIONS

These findings have demonstrated that a large proportion of the military population are susceptible to HAV infection. There are large sub-groups in which the prevalence is below 25 %. The decline in the incidence of infections in childhood and an increase in the proportion of the adult population who will be susceptible in the future will make the need for an active vaccination for the military even more essential.

Because of the dynamic nature of the trends in the incidence of HAV infection in the Israeli population, there is a need to carry out regular seroepidemiologic studies in the military population. In this way planning of future HAV vaccine trials would be greatly improved.

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9.0 TABLES

TABLE 1. Prevalence of anti-hepatitis A antibody by sex in a sample of military recruits in Israel aged 18-19 examined in 1987/8

Sex	Tested	Positive	Positive
	N	n	%
Males	440	226	51.4
Females	351	155	44.2
Total	794	382	48.1
	RR = 1.16	P = 0.045	

TABLE 2. Prevalence of anti-hepatitis A antibody by sex and ethnic origin in a sample of military recruits in Israel aged 18-19 examined in 1987/8

	Easterners			Westerners				
Sex	Tested	Positive		Tested	Positive			
	N	n	%	N	n	%	RR	P
Males	238	145	60.9	167	61	36.5	1.67	< 0.001
Females	169	90	53.3	159	56	35.2	1.51	0.001
Total	410	236	57.6	326	117	35.9	1.60	< 0.001
		RR = 1.14			RR = 1.04			
		P = 0.128			P = 0.818			

TABLE 3. Prevalence of antibody against hepatitis A virus by ethnic origin (by father) and years of education among males in a sample of military recruits in Israel aged 18-19 examined in 1987/8

Years of education	Easterners			Westerners				
	Tested	Positive		Tested	Positive			
	N	n	%	N	n	%	RR	P
<12	93	62	66.7	21	10	47.6	1.40	0.134
≥12	145	83	57.2	146	51	34.9	1.64	< 0.001
Total	238	145	60.9	167	61	36.5	1.67	< 0.001
	RR = 1.17			RR = 1.36				
	P = 0.174			P = 0.333				

TABLE 4. Prevalence of antibody against hepatitis A virus by ethnic origin (by father) and years of education among females in a sample of military recruits in Israel aged 18-19 examined in 1987/8

Years of education	Easterners			Westerners				
	Tested	Positive		Tested	Positive			
	N	n	%	N	n	%	RR	P
<12	21	11	52.4	12	6	50.0	1.05	1.000
≥12	148	79	53.4	147	50	34.0	1.57	0.001
Total	169	90	53.3	159	56	35.2	1.51	0.001
	RR = 0.98			RR = 1.47				
	P = .990			P = 0.347				

TABLE 5. Prevalence of antibody against hepatitis A virus by ethnic origin (by father) and socioeconomic status in a sample of military recruits in Israel aged 18-19 examined in 1987/8.

	Easterners			Westerners				
	Tested	Positive		Tested	Positive			
SES	N	n	%	N	n	%	RR	P
Low	205	137	66.8	74	38	51.4	1.30	0.020
High	206	99	48.1	251	79	31.5	1.53	< 0.001
Total	411	236	57.4	325	117	36.0	1.59	< 0.001
RR		1.39			1.63			
P		< 0.001			0.002			

TABLE 6. Variables associated with the presence of anti-hepatitis A antibodies in multiple logistic regression analysis in a sample of military recruits in Israel aged 18-19 examined in 1987/8.

Variable	Beta	T	P
Age ($\leq$18 = 0)	0.0116	0.32	0.751
Sex (Male = 0)	-0.0332	-0.90	0.367
Origin (East = 0)	-0.1625	-4.31	< 0.001
Education (<12 = 0)	-0.0193	0.35	0.724
SES (Low = 0)	-0.1947	-4.45	< 0.001

10.0 QUESTIONNAIRE

Demographic information

1. Age !__!__!
2. Sex !__!
3. Country of birth !__!__!__!
4. Father's country of birth !__!__!__!
5. Paternal grandfather' country of birth !__!__!__!
6. Years of education !__!__!
7. Measure of socioeconomic status !__!__!

Laboratory results

Anti-HAV antibodies 1. Positive 2. Negative !__!